Effects of water and nitrogen on growth and relative competitive ability of introduced versus native C$_4$ grass species in the semi-arid Loess Plateau of China

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Abstract: Switchgrass is an introduced C$_4$ grass in the semi-arid Loess Plateau of China, but there is a lack of information to assess its ecological invasive risk. In this study, Old World bluestems (native C$_4$ grass) and switchgrass were sowed at five mixture ratios (8:0, 6:2, 4:4, 2:6 and 0:8) under two soil water levels (80% field capacity (FC) and 40% FC) and two nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N$_0$-unfertilized and N$_1$-fertilized treatments, respectively) in a pot experiment in 2012. Biomass, root morphological traits and relative competitive abilities of these two species were analyzed. Results showed that biomass of both species was significantly greater under 80% FC or N fertilization, and switchgrass had a relatively larger root:shoot ratio (RSR). Total root length (TRL) and root surface area (RSA) of switchgrass were significantly higher under 80% FC irrespective of N treatment, while those of Old World bluestems were only significantly higher under N fertilization. N had no significant effect on TRL and RSA of switchgrass, while RSA of Old World bluestems significantly increased under 80% FC and N fertilization. Under 40% FC and N$_0$-unfertilized treatment, the aggressivity of Old World bluestems was larger than zero at 2:6 and 4:4 mixture ratios of two species, whereas it was close to zero at 6:2 mixture ratio. Root competitive ability of switchgrass significantly increased under 80% FC or N fertilization. The aggressivity of Old World bluestems was negative at 6:2 mixture ratio under 80% FC and N fertilization, while it was positive at 2:6 mixture ratio. Switchgrass may become more aggressive when N deposition or rainfall increases, while a proper mixture ratio with appropriate water and N management could help with grassland management in the semi-arid Loess Plateau.

Keywords: aggressivity; nitrogen deposition; relative competitive ability; root trait; water stress

1 Introduction

Developing stable and high-yield artificial grassland is important for promoting ecological environment in the semi-arid Loess Plateau, China (Zhang et al., 2007). However, precipitation is relatively low and unevenly distributed annually and seasonally in this area; and seasonal drought

occurs frequently and threatens plant growth. Besides, it is hard for the soil to accumulate organic matter because of severe soil and water erosion and low plant productivity. Water and nitrogen (N) are two limiting factors in the establishment of artificial grassland communities in the region (Shan and Xu, 2009).

The lack of distinct grass species in the Loess Plateau, especially herbaceous grass species, has led to problems of sole grass variety, low quality and low quantity. Introducing excellent grass species is a good option to enrich grass species. Switchgrass (*Panicum virgatum* L.), a perennial grass native to North America, was introduced to the Loess Plateau of China in the 1990s because of its high production, high adaptability to drought and nutrient-poor soils, and advantages in water and soil conservation. It has enriched local herbaceous grass species, and has proved to be successful in the region (Shui et al., 2010). However, introducing switchgrass as an exotic species to this area could cause biological invasion and threaten native biodiversity and local ecosystem, as it was reported to have a high invasive potential in California (Barney and DiTomaso, 2008).

The invasion stage of non-indigenous species includes transport, colonization, establishment and landscape spread with interspecific competition that is considered as one of the most important processes in the establishment stage (Theoharides and Dukes, 2007). Competitive exclusion by native plant species seems to be a major filter in excluding invasive species (Keane and Crawley, 2002), either through single strongly competitive species or a suite of species to reduce limited resources. The highly competitive ability of alien species could potentially facilitate invasion (Roy, 1990; Levine et al., 2003). Therefore, it is important to consider the competitive ability of native species and exotic species. Old World bluestems (*Bothriochloa ischaemum* Keng), one of the dominant species in the natural community in the semi-arid Loess Plateau of China, is a perennial C_4 herbaceous grass species and has a high tolerance to drought and poor soils (Xu et al., 2013). It is an ideal native species that could be used to estimate the invasive potential of switchgrass (Keane and Crawley, 2002).

As the resource hypothesis suggests, increased resource availability (light, water and soil nutrients) tends to facilitate invasion by exotic plants (Davis et al., 2000; Daehler, 2003; Blumenthal, 2005). This raises the concern of soil N availability, which is significantly enhanced by increased N deposition (Galloway et al., 2008; Bobbink et al., 2010). Many studies have proved that N deposition could facilitate the invasion of non-native species by increasing the growth and competition of non-native species more strongly than those of native species (Rao and Allen, 2010; He et al., 2011; Liu et al., 2017). Water, another limiting factor in the semi-arid Loess Plateau of China, may also affect the invasion of alien species by affecting their growth and competition to native species (Blumenthal et al., 2008; Liu et al., 2017). Therefore, it is necessary to include the effects of water and N when studying the competitive relationship between switchgrass and Old World bluestems.

Plant root morphological traits, including root hair, root mass, root length and root area, play important roles in resource acquisition (water and nutrients), which could contribute to plant growth and competitive ability (Aerts et al., 1991; Bennett et al., 2016; Ravenek et al., 2016; Semchenko et al., 2018). For example, N addition could increase the competitive ability of an invasive plant (*Solidago canadensis*) by enhancing its root biomass (Ren et al., 2019). Semchenko et al. (2018) also found that species with the lower specific root length and less branched roots tended to be better at tolerating competition. Thus, it would be interesting to include root morphological traits in the study of competition between switchgrass and Old World bluestems under variable resource supplies.

Switchgrass can be used as a biofuel and lots of studies focused on raising cellulosic ethanol production from switchgrass, either on the physiological or the molecular level (Sanderson et al., 2006; Mitchell et al., 2008; Keshwani and Cheng, 2009). Switchgrass is the ideal forage, because of its low requirements for agricultural inputs and positive environmental impacts (Ashworth et al., 2019). Its resistance to drought, heat, cold and alkaline stresses, its benefits in soil and water conservation, nutrient recovery from runoff and potential for carbon sequestration were also studied (Muir et al., 2001; Xu et al., 2003; Cooney et al., 2017; Collins et al., 2020). However, few studies are assessing the relative competitive abilities of switchgrass and local herbaceous

grasses in the semi-arid Loess Plateau of China, and how this ability differed as to the changes of mixture ratio, water and N supply.

Given that switchgrass was relatively poorly adapted to drought and its biomass was less responsive to N addition compared with Old World bluestems (Xu et al., 2003), and increased resources normally favor exotic plants to reproduce (Davis et al., 2000). We hypothesized that: (1) increased water and/or N supply would improve the growth of Old World bluestems and switchgrass; (2) the competitive ability of switchgrass would be weaker than Old World bluestems under low water and N conditions; and (3) increased water and/or N supply would increase competitive ability of switchgrass to Old World bluestems.

2 Materials and methods

2.1 Plant materials

Old World bluestems and switchgrass were used in this experiment. Seeds of Old World bluestems were collected in October, 2011 from the experimental fields at Ansai Research Station (ARS) of the Chinese Academy of Sciences (CAS) (36°51′N, 109°19′E; 530 m a.s.l.), located at the center of semi-arid hilly gully region on the Loess Plateau, China. The seeds were stored in a sealed container of the laboratory under natural dry conditions. The variety of switchgrass is Alamo, and the seeds were also collected from the same place in October, 2005.

2.2 Growth conditions

2.2.1 Soil property

The soil was collected from the upper 20 cm of a farm field at ARS, CAS. The soil moisture content at field capacity (FC) was 0.20 g/g. Soil pH (10 g dry soil in the 25 mL water) was 8.77, and soil organic matter content, soil total N, total phosphorus (P), and total potassium (K) contents were 0.360%, 0.025%, 0.066% and 1.900%, respectively. Soil available N, P and K contents were 19.6, 50.8 and 101.6 mg/kg, respectively. After being dried and sealed, we packed 3.8 kg soil into each cylindrical pot (20 cm in diameter and 16 cm in depth), and a vertical plastic pipe was placed adjacent to the inner wall of each pot for water supply. The experiment was conducted under a rainfall shelter at ARS, CAS from April to November, 2012. The annual mean temperature was 12.9°C, the maximum mean temperature was 26.7°C in July, and the minimum temperature from –1.0°C to –2.0°C in January. The mean annual precipitation was 638 mm.

2.2.2 Soil FC

Soil FC was determined as follows: (1) three 100-cm^3 core samples were filled with the disturbed soil at a bulk density of 1.2 g/cm^3; (2) when core samples were saturated, they were placed on a coarse sand soil to drain water freely, and the surface was covered to prevent evaporation; (3) soil wet mass (m_w) was determined until the weight of soil core was constant (i.e., two measurements differed <0.20 g). After that, core samples were dried in an oven at 105°C for 48 h and dry soil mass (m_d) was calculated; and (4) FC was calculated by the following equation: FC=(m_w–m_d)/m_d.

2.2.3 Mixture ratio of species

A replacement series design (de Wit, 1960; Jolliffe, 2000) was used with a density of 8 plants per pot. Five mixture ratios of Old World bluestems (B) to switchgrass (P) (8:0, 6:2, 4:4, 2:6 and 0:8) were used (Fig. 1). The ratios of 8:0, 6:2, 4:4, 2:6, and 0:8 for these two species were abbreviated as B8P0, B6P2, B4P4, B2P6 and B0P8 in the following figures.
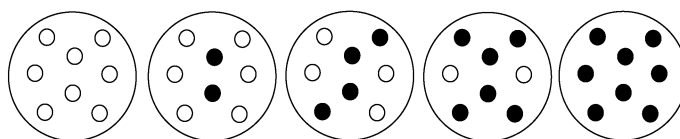

Fig. 1 Schematic diagram of the experimental design. Open and filled circles represent Old World bluestems and switchgrass, respectively.

2.2.4　N treatment

Two N fertilization rates (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively) were applied in this experiment. N was applied as $CO(NH_2)_2$ (urea) and mixed with soil uniformly during pot filling. There were three replicates for each treatment, and all pots were distributed in a completely random design inside the rainfall shelter.

2.2.5　Water treatment

Seeds were sown on 3 April and soil water content was maintained at 80% FC during the seedling establishment. When the seedlings of both species had three leaves and were approximately 0.10 m high (25 d after sowing), seedlings were thinned to 8 plants per pot, and water treatments (40% FC and 80% FC) started. Before the water treatment, a layer of perlite was spread on the soil surface of each pot (approximately 2.0 cm) to reduce soil evaporation. Daily evapotranspiration was assessed by weighing the pots at 18:00 (LST) daily, and water was added via the plastic pipes to maintain the desired level.

2.3　Biomass

Plants were harvested at the end of growing season on October 5, 2012 for both species. Shoots were collected and separated into leaf and stem, and roots were collected and carefully washed free of soil. Weights of each part were determined after drying at 75°C for 72 h in an oven. Root:shoot ratio (RSR) was calculated.

2.4　Root morphological trait

About 1/3 of total roots were subsampled for morphological trait measurements. The selected root samples were dyed with 0.5% methylene blue solution for 5 min, gently dried with a paper tissue, and put between two transparent plastic sheets. Then, all samples were scanned by a BENQ color scanner 5560 (BENQ Science and Technology Ltd., Shanghai, China) (Xu et al., 2012; Wang et al., 2018) and analyzed by WinRHIZO 2009 (Regents Instruments Canada lnc., Quebec City, Quebec, Canada) to determine total root length (TRL), root surface area (RSA) and root average diameter (RAD).

2.5　Water use efficiency (WUE)

All pots were weighted and watered at 18:00, and the water added was recorded. The pots filled with soil but without plants were used to estimate soil evaporation. Soil evaporation was subtracted from total water consumption. WUE was defined as the amount of total biomass produced per unit of water consumed by plants under each water and N treatment (WUE, g DW/kg H_2O). The formula is as follow:

$$WUE=total\ biomass/(total\ water\ consumption–soil\ evaporation). \tag{1}$$

2.6　Competitive index

Competitive index is a useful tool for estimating the effect and intensity of competition (Goldberg et al., 1999), and there are two indices that are commonly used (de Wit and Van den Bergh, 1965; Jolliffe, 2000). Aggressivity (A) is considered as an appropriate expression for the intensity of interspecific competition, while relative yield total (RYT) is commonly used in measuring the effect of competition (McGilchrist and Trenbath, 1971; Grace, 1995; Weigelt and Jolliffe, 2003).

These two competitive indices are calculated as follows:

$$A=Y_{ab}/(Y_{aa}\times Z_{ab}) -Y_{ba}/(Y_{bb}\times Z_{ba}), \tag{2}$$

$$RYT=Y_{ab}/Y_{aa}+Y_{ba}/Y_{bb}, \tag{3}$$

where Y_{ab} is the biomass of Old World bluestems in the mixture (g/pot); Y_{aa} is the biomass of Old World bluestems in monoculture (g/pot); Z_{ab} is the ratio of Old World bluestems to switchgrass in the mixture; Y_{ba} is the biomass of switchgrass in the mixture (g/pot); Y_{bb} is the biomass of switchgrass in monoculture (g/pot); and Z_{ba} is the ratio of switchgrass to Old World bluestems in the mixture.

For the index of A, A<0 indicates Old World bluestems is less competitive than switchgrass; A=0 indicates Old World bluestems is similarly competitive with switchgrass; and A>0 indicates

Old World bluestems is more competitive than switchgrass.

For the index of RYT, RYT=1.0 indicates that there is a competition between two species; RYT<1.0 indicates that there is a mutual antagonism between two species; and RYT>1.0 indicates that there is a niche differentiation between two species (Hector, 1998).

2.7 Statistical analysis

Generalized least square models were used to test the differences in shoot biomass, root biomass, total biomass, WUE, TRL, RSA, RAD, A and RYT among water levels, N treatments, mixture ratios and their interactions. The residuals of each model were visually checked for heteroscedasticity and in the presence of heteroscedasticity, and we specified appropriate variance structures based on Akaike Information Criterion if they significantly improved the model (Zuur et al., 2009). Data and statistical analyses were performed using R software platform (R Core Team, 2019). The effect package was used to determine means, standard errors and 95% confidence intervals (Fox, 2003). The Multcomp package was used to do Tukey's post hoc comparisons and define differences at P=0.05 level.

3 Results

3.1 Biomass and RSR

Water, N and mixture ratios significantly affected the growth and RSR of Old World bluestems and switchgrass (Table 1). Regardless of water, the biomass of both species under N fertilization was significantly higher than that under no N fertilization. The biomass of both species was also significantly higher under 80% FC than under 40% FC irrespective of N treatment. The shoot, root and total biomass of each individual species decreased as its corresponding proportion decreased in the mixture (Figs. 2 and 3).

Table 1 Analysis of variance of the effects of soil water, nitrogen, mixture ratio and their interactions on shoot, root and total biomass, root:shoot ratio (RSR) and water use efficiency (WUE) of Old World bluestems (B) and switchgrass (P)

Index	Species	Water (W)	Nitrogen (N)	Mixture ratio (MR)	W×N	W×MR	N×MR	W×N×MR
Shoot biomass	B	<0.001	<0.001	<0.001	<0.001	0.422	0.001	0.776
	P	<0.001	<0.001	<0.001	<0.001	0.257	<0.001	0.402
	B+P	<0.001	<0.001	<0.001	<0.001	0.031	<0.001	0.492
Root biomass	B	0.001	<0.001	<0.001	0.007	0.218	<0.001	0.782
	P	0.003	<0.001	<0.001	0.045	0.176	0.356	0.285
	B+P	<0.001	<0.001	<0.001	0.002	0.291	<0.001	0.477
Total biomass	B	<0.001	<0.001	<0.001	0.470	0.914	<0.001	0.264
	P	<0.001	<0.001	<0.001	0.386	0.529	0.321	0.744
	B+P	<0.001	<0.001	0.160	0.870	0.434	0.691	0.724
RSR	B	<0.001	<0.001	<0.001	<0.001	0.670	<0.001	0.588
	P	<0.001	<0.001	<0.001	<0.001	0.346	<0.001	0.660
	B+P	<0.001	<0.001	<0.001	<0.001	0.378	<0.001	0.673
WUE	B+P	<0.001	<0.001	<0.001	0.020	0.646	0.727	0.863

Total shoot biomass in the mixture was significantly lower than that of Old World bluestems in monoculture, and higher than that of switchgrass in monoculture. Total root biomass in the mixture was significantly lower than those of both species in monoculture (Fig. 2). Total biomass in the mixture was significantly lower than that of Old World bluestems in monoculture, while it was higher than that of switchgrass in monoculture only within N fertilization (Fig. 3).
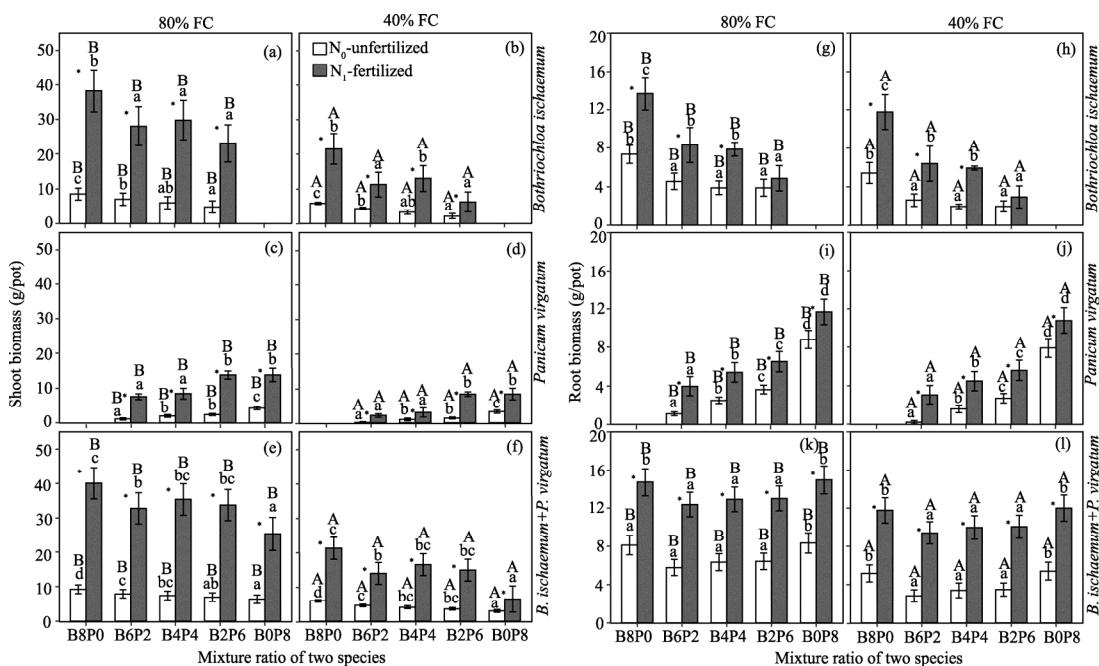
Fig. 2 Shoot (a–f) and root biomass (g–l) of Old World bluestems (*Bothriochloa ischaemum*) and switchgrass (*Panicum virgatum*) under different soil water levels (40% FC and 80% FC), nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively) and mixture ratios of two species. Bars are standard errors. Different uppercase letters indicate significant differences between two soil water levels within each mixture ratio and N treatment, while different lowercase letters indicate significant differences among different mixture ratios within each soil water level and N treatment. * indicates significant differences between two N treatments within each mixture ratio and soil water level (based on Tukey's post hoc analysis, $P<0.05$ level). B8P0, B6P2, B4P4, B2P6 and B0P8 indicate 8:0, 6:2, 4:4, 2:6 and 0:8 mixture ratios of Old World bluestems to switchgrass, respectively. The abbreviations are the same as in Figures 3–6.
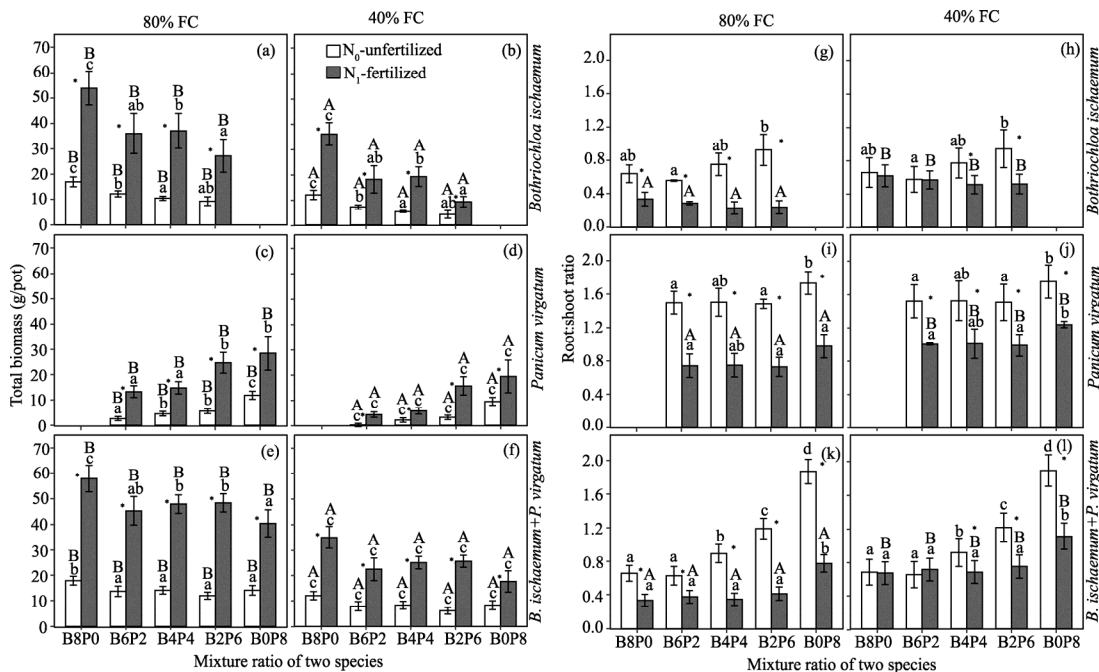
Fig. 3 Total biomass (a–f) and root:shoot ratio (g–l) of Old World bluestems (*Bothriochloa ischaemum*) and switchgrass (*Panicum virgatum*) under different soil water levels (40% FC and 80% FC), nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively) and mixture ratios.

Bars are standard errors.

RSR of Old World bluestems and switchgrass under N fertilization was generally significantly lower than those under no N fertilization irrespective of water supply. RSR of both species was also significantly lower under 80% FC than under 40% FC for N fertilization, while there was no significant difference between water levels for no N fertilization. RSR of switchgrass was significantly lower in the mixture than in monoculture, whereas RSR of Old World bluestems was significantly higher in the mixture than in monoculture for no N fertilization (Fig. 3).

3.2 Root morphological trait

The mixture of two species did not have any significant effects on TRL and RSA of Old World bluestems and switchgrass (Table 2). TRL and RSA of switchgrass were significantly higher under 80% FC than under 40% FC irrespective of N treatment, while those of Old World bluestems were only significantly higher under 80% FC than under 40% FC for no N fertilization (Fig. 4). N showed no significant effect on TRL and RSA of switchgrass, and RSA of Old World bluestems was significantly higher under N fertilization for 80% FC. However, TRL and RSA of Old World bluestems were significantly lower under N fertilization for 40% FC (Fig. 4).

Table 2 Analysis of variance of the effects of soil water, nitrogen, mixture ratio and their interactions on total root length, root surface area and root average diameter of Old World bluestems (B) and switchgrass (P)

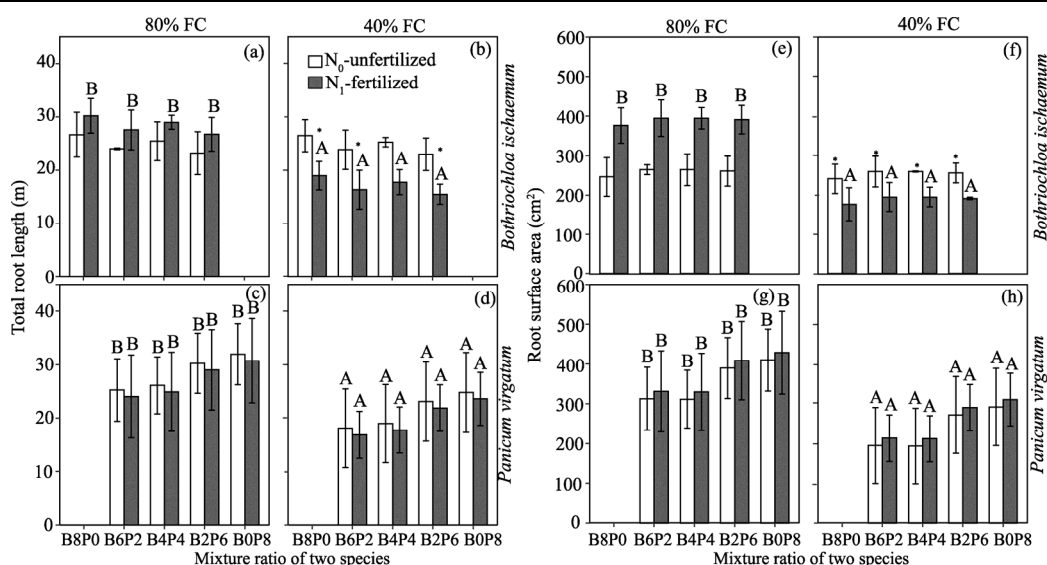| Source of variation	df	Total root length		Root surface area		Root average diameter	
		B	P	B	P	B	P
Water (W)	1	<0.001	0.036	<0.001	0.009	0.263	0.001
Nitrogen (N)	1	<0.001	0.012	0.232	0.073	<0.001	<0.001
Mixture ratio (MR)	3	<0.001	0.060	<0.001	0.022	0.313	0.030
W×N	1	<0.001	0.294	<0.001	0.271	0.014	0.958
W×MR	3	0.142	0.160	0.212	0.140	0.020	0.057
N×MR	3	0.531	0.109	0.507	0.091	0.667	0.519
W×N×MR	3	0.775	0.203	0.742	0.191	0.070	0.847

Fig. 4 Total root length (a–d) and root surface area (e–h) of Old World bluestems (*Bothriochloa ischaemum*) and switchgrass (*Panicum virgatum*) under different soil water levels (40% FC and 80% FC), nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively) and mixture ratios. Bars are standard errors.

The mixture of two species had no significant effects on RAD of Old World bluestems. RAD of switchgrass varied among mixture ratios, with the largest at B2P6 under 40% FC (Fig. 5). In

monoculture, RAD of Old World bluestems was significantly larger under 80% FC for no N fertilization, while it showed an opposite trend for N fertilization. Compared with 40% FC, RAD of switchgrass was always larger under 80% FC, except that at B2P6. RAD of Old World bluestems in monoculture and B2P6 was significantly larger under N fertilization for 40% FC, whereas RAD of switchgrass from all the mixture ratios was always larger under N fertilization (Fig. 5).
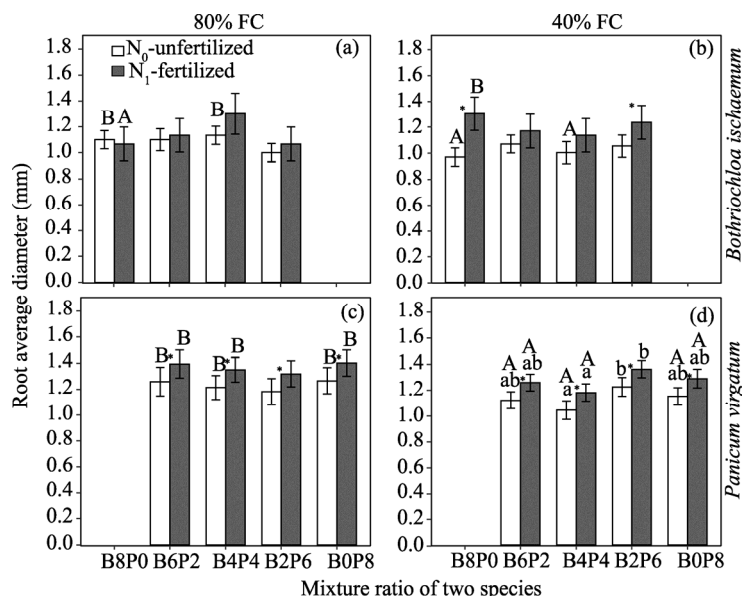

Fig. 5 Root average diameter (RAD) of Old World bluestems (*Bothriochloa ischaemum*, a and b) and switchgrass (*Panicum virgatum*, c and d) under different soil water levels (40% FC and 80% FC), nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively) and mixture ratios. Bars are standard errors.

3.3 Competitive index

Mixture, water and N significantly affected the values of A of Old World bluestems compared with switchgrass (Table 3). The sequence of A values calculated from shoot, root and total biomass showed as B6P2≤B4P4≤B2P6. The values of A from shoot, root and total biomass were close to zero at B6P2 under 40% FC and no N fertilization, while they were negative under 80% FC and/or N fertilization (Table 4).

The value of A from shoot biomass under 80% FC was significantly lower than that under 40% FC within each mixture ratio and no N fertilization, while they were significantly higher at B4P4 and B2P6 under N fertilization. The value of A from shoot biomass under N fertilization was lower for 40% FC, and the difference was only significant at B6P2. Within 80% FC, the values of A from shoot biomass at B4P4 and B2P6 were significantly higher under N fertilization than under no N fertilization, while the value of A from shoot biomass at B6P2 showed an opposite trend (Table 4).

The value of A from root biomass at B6P2 was significantly lower under 80% FC than under 40% FC with no N fertilization, while water had no significant effect on those values at B4P4 and B2P6. The values of A from root biomass at B6P2 and B4P4 were significantly lower under 80% FC than under 40% FC with N fertilization, whereas the value of A from root biomass at B2P6 showed no significant difference between different water levels. The values of A from root biomass at B6P2 and B2P6 were significantly lower under N fertilization with 40% FC, while N had no significant effect at B4P4. The values of A from root biomass at B6P2 and B4P4 were significantly lower under N fertilization with 80% FC, while N had no significant effect at B2P6 (Table 4).

The value of A from total biomass of each mixture ratio was significantly lower under 80% FC

than under 40% FC with no N fertilization. The value of A from total biomass at B6P2 was also significantly lower under 80% FC than under 40% FC with N fertilization, while it showed an opposite trend for B2P6. The values of A from total biomass at B6P2 and B2P6 were significantly lower under N fertilization with 40% FC, while there was no significant difference at B4P4. The value of A from total biomass at B2P6 was significantly higher under N fertilization with 80% FC, whereas it showed an opposite trend at B6P2 (Table 4).

Table 3 Analysis of variance for the effects of soil water, nitrogen, mixture ratio and their interactions on the aggressivity and relative yield total

Source of variation	df	Aggressivity (A)			Relative yield total (RYT)		
		Shoot	Root	Total biomass	Shoot	Root	Total biomass
Water (W)	1	0.002	0.543	<0.001	<0.001	0.035	<0.001
Nitrogen (N)	1	0.019	<0.001	<0.001	<0.001	<0.001	<0.001
Mixture ratio (MR)	2	<0.001	<0.001	<0.001	<0.001	0.398	0.104
W×N	1	0.056	<0.001	0.002	<0.001	0.414	0.414
W×M	2	0.045	0.290	0.080	0.099	0.275	0.275
N×M	2	<0.001	<0.001	0.142	0.133	0.264	0.264
W×N×MR	2	0.007	0.032	0.059	0.557	0.804	0.804

Table 4 Aggressivity values of shoot, root and total biomass of Old World bluestems (B) to switchgrass (P) under different water and nitrogen (N) treatments

Treatment	Mixture ratio	Shoot	Root	Total biomass
N_0-unfertilized+80% FC	B6P2	−0.23±0.12[aA*]	−0.14±0.02[aA*]	−0.17±0.01[aA*]
	B4P4	−0.26±0.25[abA*]	0.32±0.01[b*]	−0.03±0.25[aA]
	B2P6	0.36±0.06[bA*]	1.07±0.30[c]	0.48±0.01[bA*]
N_1-fertilized+80% FC	B6P2	−0.79±0.20[a*]	−0.93±0.17[aA*]	−1.25±0.02[aA*]
	B4P4	1.16±0.22[bB*]	−0.01±0.08[bA*]	0.01±0.16[b]
	B2P6	4.45±1.47[bB]	1.06±0.43[b]	1.32±0.44[cB*]
N_0-unfertilized+40% FC	B6P2	0.06±0.07[aB*]	0.02±0.10[aB*]	0.05±0.05[aB*]
	B4P4	0.34±0.18[abB]	0.13±0.04[a]	0.24±0.07[aB]
	B2P6	1.08±0.25[bB]	1.11±0.34[b*]	1.10±0.21[bB*]
N_1-fertilized+40% FC	B6P2	−0.59±0.17[a*]	−0.43±0.11[aB*]	−0.51±0.11[aB*]
	B4P4	0.30±0.27[bA]	0.25±0.04[bB]	0.28±0.14[b]
	B2P6	0.56±0.11[bA]	0.49±0.17[b*]	0.53±0.11[bA*]

Notes: Different lowercase letters indicate significant differences among mixture ratios under each water level (40% FC and 80% FC) and N treatment (0 and 100 mg N/kg dry soil, termed N_0-unfertilized and N_1-fertilized treatments, respectively), while different uppercase letters indicate significant differences between two soil water levels within each mixture ratio and N treatment, and * indicates significant differences between two N treatments within each mixture ratio and soil water level (based on Tukey's post hoc analysis, $P<0.05$ level). Mean±SE; n=3.

RYT values of Old World bluestems and switchgrass were significantly affected by the mixture ratios (Table 3), with RYT values from shoot biomass being in the order of B6P2=B4P4>B2P6 (Table 5). RYT values under 80% FC or N fertilization were significantly higher than those under 40% FC and N_0-unfertilized, and RYT values under N fertilization and 40% FC were higher than those under N_0-unfertilized and 80% FC. Under 40% FC and N_0-unfertilized, RYT values from shoot, root and total biomass under each mixture ratio and water supply were all smaller than 1, while those values were all above 1 (the highest) under 80% FC and N fertilization. Besides, RYT values from shoot biomass were greater than those from root and total biomass with N and water supply (Table 5).

Table 5 Relative yield total of shoot, root and total biomass of Old World bluestem (B) and switchgrass (P) under different water and nitrogen (N) treatments

Treatment	Mixture ratio	Shoot	Root	Total biomass
N$_0$-unfertilized+80% FC	B6P2	0.83±0.02^{bA*}	0.72±0.06^{B*}	0.79±0.03^{bB*}
	B4P4	0.89±0.05^{bA*}	0.75±0.06^{B*}	0.86±0.03^{bB*}
	B2P6	0.74±0.01^{aA*}	0.82±0.06^{B*}	0.70±0.02^{aB*}
N$_1$-fertilized+80% FC	B6P2	2.31±0.12^{bB*}	0.93±0.06^{B*}	1.09±0.07^{aB*}
	B4P4	2.37±0.12^{bB*}	0.96±0.06^{B*}	1.17±0.08^{aB*}
	B2P6	2.22±0.12^{aB*}	1.02±0.06^{B*}	1.43±0.09^{bB*}
N$_0$-unfertilized+40% FC	B6P2	0.91±0.03^{bB*}	0.60±0.06^{A*}	0.72±0.05^{bA*}
	B4P4	0.97±0.05^{bB*}	0.63±0.06A^*	0.78±0.06^{bA*}
	B2P6	0.81±0.02^{aB*}	0.69±0.06^{A*}	0.63±0.05^{aA*}
N$_1$-fertilized+40% FC	B6P2	1.06±0.11^{bA*}	0.81±0.06^{A*}	0.81±0.06^{aA*}
	B4P4	1.11±0.11^{bA*}	0.84±0.06^{A*}	0.89±0.06^{aA*}
	B2P6	0.96±0.11^{aA*}	0.90±0.06^{A*}	1.15±0.05^{bA*}

Notes: Different lowercase letters indicate significant differences among mixture ratios under each water level (40% FC and 80% FC) and N treatment (0 and 100 mg N/kg dry soil, termed N$_0$-unfertilized and N$_1$-fertilized treatments, respectively), while different uppercase letters indicate significant differences between two soil water levels within each mixture ratio and N treatment, and * indicates significant differences between two N treatments within each mixture ratio and soil water level (based on Tukey's post hoc analysis, $P<0.05$ level). Mean±SE; n=3.

3.4 WUE

WUE values of Old World bluestems and switchgrass were significantly lower under 80% FC than under 40% FC. However, WUE values of Old World bluestems and switchgrass were significantly higher under N fertilization with 80% FC. Besides, WUE values of Old World bluestems and switchgrass in the mixture were always lower than that of Old World bluestems in monoculture, while it showed no significant difference with that of switchgrass in monoculture (Fig. 6).
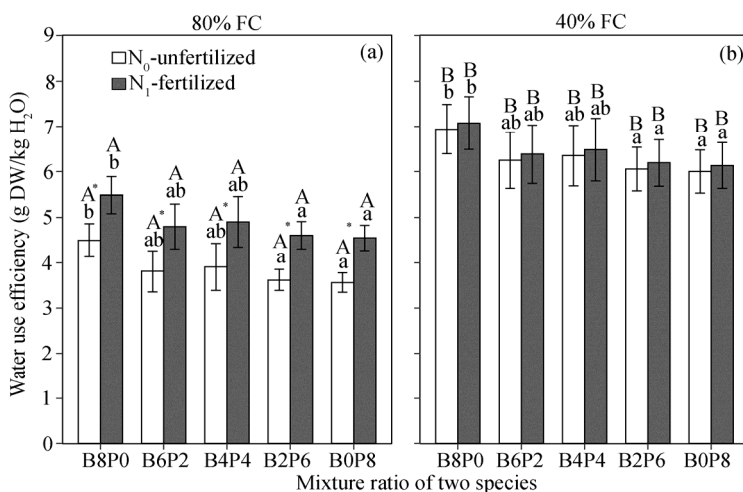

Fig. 6 Water use efficiency (WUE; a and b) of Old World bluestems (*Bothriochloa ischaemum*) and switchgrass (*Panicum virgatum*) together under different soil water levels (40% FC and 80% FC), nitrogen (N) treatments (0 and 100 mg N/kg dry soil, termed N$_0$-unfertilized and N$_1$-fertilized treatments, respectively) and mixture ratios. Bars are standard errors.

4 Discussion

Water and N are two most limiting factors in the semi-arid ecosystem (Hooper and Johnson, 1999; Yang et al., 2011). Water deficit could restrict the height, tillering number and biomass of plant

species, while N addition could alleviate these negative effects by preventing cell membrane damage and enhancing osmoregulation of plant species (Li et al., 2000; Guo et al., 2010; Abid et al., 2016) or strengthen those negative effects (Dziedek et al., 2016). Our results confirmed that water and N co-limited the growth of Old World bluestems and switchgrass, and N fertilization could alleviate the negative effects of water deficiency.

Water and N availability could also affect plant competitive ability (Rao and Allen, 2010; He et al., 2011; Sheppard et al., 2014). Aggressivity is an important index to decide the competitive abilities of different plant species, while RYT could be used to judge if there are complementary effect between two species in the mixture (Fowler, 1982; Weigelt and Jolliffe, 2003). Our study showed that under low water and N_0-unfertilized, aggressivity of Old World bluestems to switchgrass was bigger or close to zero, which agrees with our hypothesis that the competitive ability of switchgrass is weaker than Old World bluestems under low water and N_0-unfertilized. Compared with switchgrass, Old World bluestems has relatively better adaptability to drought and nutrient-poor soils (Xu et al., 2003), and the competitive ability of switchgrass was restricted in this environment. Similarly, a meta-study showed that drought could potentially inhibit the growth of invasive species more than the native species, and then reduced plant invasion (Liu et al., 2017). However, the competitive ability of Old World bluestems to switchgrass generally decreased as singularly water or N supply improved. The higher competitive ability of Old World bluestems can be attributed to the adaption of local environment with the low soil water and nutrient. When facing more available water, nutrient or variable environment, Old World bluestems is recalcitrant to the change (Xu et al., 2013). These results agree with the resource hypothesis that invasive species outperformed native species in high resource environments (Davis et al., 2000; Daehler, 2003; Blumenthal, 2005; Liu et al., 2017). This may put Old World bluestems at risk under enhanced N deposition conditions (Liu et al., 2013).

The outperformance of switchgrass under 80% FC and N fertilization could be explained by its high resource-acquisition efficiency. Root morphology could reflect plant growth and their resource exploiting strategy, and longer root length and larger RSA are always positively correlated to better water and nutrient uptake (Lynch, 2013; White et al., 2013). A high or efficient resource uptake capacity of invasive plant species could confer a competitive advantage (Funk and Vitousek, 2007; Drenovsky et al., 2008). It is also reported that invasive species could increase their competitive ability by allocating more biomass to roots (namely a higher RSR) (Aerts et al., 1991; Rajaniemi, 2002). Thus, changing biomass allocation and modifying root morphology are widely recognized as effective strategies for plants to cope with competitive pressure (Ravenek et al., 2016; Semchenko et al., 2018). In our study, switchgrass generally allocated more biomass to roots than Old World bluestems, which gives it advantages in acquiring limited soil resources. TRL and RSA of switchgrass increased under singularly higher water supply, which showed that the resource uptake capacity of switchgrass increased with more water supply. Interestingly, TRL and RSA of Old World bluestems decreased under singularly N fertilization with 40% FC, indicating a decreased resource uptake capacity of Old World bluestems with more N supply under drought condition. The changes of TRL and RSA of switchgrass and Old World bluestems, together with the higher RSR of switchgrass, could partly explain the relatively increased competitive ability of switchgrass to Old World bluestems under the higher water or N supply. It is noted that although the shoot competitive ability of Old World bluestems to switchgrass was very strong, the root competitive ability of switchgrass was stronger, which resulted in a stronger full (root and shoot) competitive ability of switchgrass to Old World bluestems. This also agrees with the general belief that the effect of the belowground competition is greater than shoot competition, and it could determine which species or individuals dominate (Wilson, 1988; Kiær et al., 2013).

Mixture ratio or planting density could also affect the result of competition. For example, it is shown that a low wood density is generally correlated with a low competitive effect on neighbors (Kunstler et al., 2016). However, the competitive result in our study is opposite, i.e., the competitive ability of Old World bluestems to switchgrass was stronger with a lower ratio of Old World bluestems (B6P2≤B4P4≤B2P6). This suggested that plant density could not simply be used to explain the competitive result. Interestingly, although singular water or N supply generally

improved the relative competitive ability of switchgrass, the competitive ability of Old World bluestems at B2P6 was the highest under 80% FC and N fertilization, and there is a niche differentiation between switchgrass and Old World bluestems. This is probably because the niche differentiation is different among various mixture ratios, and water and N supply could further enhance it.

We are experiencing a world with global climate change, including spatiotemporal variation of extreme precipitation. General circulation model results showed that more frequent and intense precipitation will occur in the Loess Plateau, China (Li et al., 2012), which could increase soil water content in a short term. Besides, a TRMM (the Tropical Rainfall Measuring Mission) multi-satellite precipitation data analysis showed that the average precipitation in the Loess Plateau is increasing significantly at the rate of 4.46 mm/a (Zhao et al., 2018). N deposition is also increasing in China (Liu et al., 2013; Yu et al., 2019). From our study result and the impact of climate change on the Loess Plateau, we speculate that the invasion potential of switchgrass will increase in the future. Our recent study showed increased water supply increased the aggressivity of Old World bluestems to *Lespedez adavurica* (Laxm.) Schindl. (a perennial C_3 leguminous sub-shrub in the Loess Plateau; unpublished data). This also suggests that the aggressivity of switchgrass to *L. adavurica* might be greater under increasing precipitation. Ecologists and farmers should be aware of the potential for switchgrass to increase its invasion potential in the Loess Plateau under increasing precipitation or N deposition.

5 Conclusions

Either increased water or N supply had a positive effect on the biomass production of Old World bluestems and switchgrass, and N fertilization could alleviate the negative effects of water stress on biomass production. Old World bluestems was relatively dominant under 40% FC and no N application, when water supply or N condition was improved, switchgrass became more aggressive. Root traits partly explain the change of relative competitive ability of switchgrass to Old World bluestems under increased water or N supply. However, a suitable mixture ratio with an optimal water and N-fertilizer management could prevent the invasion of switchgrass. This means, when N deposition or precipitation increases in the arid and semi-arid area and the biological invasion potential of alien species increases, we can adopt suitable practices, like changing species combination ratio, water or nitrogen supply to reduce the risk of invasion. Although the transfer of the results from a pot experiment on plant growth and competition to field conditions needs to be cautious, the information from this study may help in predicting the risk of introduced invasive species.

Acknowledgements

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